

tific) was used as the medium. The glucose concentration in the medium was 5 g/L. DIV6 and after, "Complete Brainpys Medium" (product name) (CDI) was used as the medium. The glucose concentration in the medium was 0.5 g/L.

[0053] The medium was replaced as follows. First, 250 μ L, 150 μ L or 50 μ L of medium was aspirated from the culture container at one time. Subsequently, a new medium having the same capacity as the aspirated medium was added. The medium was replaced three times a week. The aspirated medium was used to measure a glucose concentration. FLEX2 (Nova Biomedical Co., Ltd.), which is a medium component analyzer, was used for measuring the glucose concentration.

[0054] FIG. 1 is a graph showing results of measuring changes over time in glucose concentration in a medium. A horizontal axis shows the number of culture days from seeding of nerve cells, and a vertical axis shows the glucose concentration (g/L) in the medium.

[0055] As a result, it was clarified that the glucose concentration in the medium was maintained at the highest level in a case where 50 μ L of the medium once was replaced three times a week. When 50 μ L of medium once was replaced, the glucose concentration in the medium on the 22nd day after seeding the nerve cells was about 1.4 g/L. In addition, the glucose concentration in the medium on 40th day after seeding the nerve cells was about 0.2 g/L.

[0056] On the other hand, in a case where 150 μ L of medium once was replaced three times a week, the glucose concentration in the medium on the 22nd day after seeding the nerve cells was about 0.4 g/L. In addition, the glucose concentration in the medium on 40th day after seeding the nerve cells was about 0 g/L.

[0057] In addition, in a case where 250 μ L of medium once was replaced three times a week, the glucose concentration in the medium on the 22nd day after seeding the nerve cells was about 0.3 g/L. In addition, the glucose concentration in the medium on 40th day after seeding the nerve cells was about 0 g/L.

Experimental Example 2

[0058] (Examination of Aggregation Level of Nerve Cells)

[0059] Each nerve cell cultured in Experimental Example 1 was observed with a microscope, and an aggregation level thereof was evaluated. Evaluation criteria for the aggregation level were as follows. As the aggregation level increased, the adhesion area between the nerve cells and the culture surface became smaller and the nerve cells tended to be separated from the culture surface.

[0060] <<Evaluation Criteria for Aggregation Level>>

[0061] 1: Adhesion area between the nerve cells and the culture surface was 3.14 mm² or more and 28.2 mm² or less per 80,000 nerve cells.

[0062] 2: Adhesion area between the nerve cells and the culture surface was 0.949 mm² or more and less than 3.14 mm² per 80,000 nerve cells.

[0063] 3: Adhesion area between the nerve cells and the culture surface was 0.196 mm² or more and less than 0.949 mm² per 80,000 nerve cells.

[0064] 4: Adhesion area between the nerve cells and the culture surface was 0 mm² or more and less than 0.196 mm² per 80,000 nerve cells.

[0065] FIGS. 2A to 2C show representative micrographs of each nerve cell at 53rd day (DIV53) from seeding of

nerve cells. FIG. 2A shows a micrograph of nerve cells obtained by replacing 250 μ L of the medium once three times a week. FIG. 2B shows a micrograph of nerve cells obtained by replacing 150 μ L of the medium once three times a week. FIG. 2C shows a micrograph of nerve cells obtained by replacing 50 μ L of the medium once three times a week.

[0066] As a result, the aggregation level of the nerve cells on DIV53 obtained by replacing 250 μ L of the medium once three times a week was 4. In addition, the aggregation level of the nerve cells on DIV53 obtained by replacing 150 μ L of the medium once three times a week was 3. In addition, the aggregation level of the nerve cells on DIV53 obtained by replacing 50 μ L of the medium once three times a week was 1.

[0067] From the results, it was clarified that the nerve cells cultured under the condition in which the glucose concentration in the medium was maintained high tended to have a low aggregation level. More specifically, it was clarified that the nerve cells having a glucose concentration of 1 g/L or higher in the medium on 22nd day after seeding the nerve cells tended to have a low aggregation level. Furthermore, it was clarified that the nerve cells having a glucose concentration of 0.2 g/L or higher in the medium on 40th day after seeding the nerve cells tended to have a low aggregation level.

Experimental Example 3

[0068] (Examination of Aggregation Level and Action Potential of Nerve Cells)

[0069] In the same manner as Experimental Example 1, nerve cells cultured for 42 days from seeding of the nerve cells was observed with a microscope, and an aggregation level thereof was evaluated. Evaluation criteria for the aggregation level were the same as those of Experimental Example 2. In addition, the action potential of each nerve cell was measured and evaluated using a microelectrode array. The evaluation criteria of the action potential were as follows, and detectability for the action potential could be detected was determined.

[0070] <<Evaluation Criteria for Action Potential>>

[0071] A: The action potential could be detected well.

[0072] B: The action potential could be detected.

[0073] C: The action potential could not be detected.

[0074] FIG. 3A shows a representative micrograph of nerve cells evaluated as Aggregation Level 1. FIG. 3B shows a representative micrograph of nerve cells evaluated as Aggregation Level 2. FIG. 3C shows a representative micrograph of nerve cells evaluated as Aggregation Level 3. FIG. 3D shows a representative micrograph of nerve cells evaluated as Aggregation Level 4. In addition, Table 1 below shows evaluation results of the aggregation level and the action potential.

TABLE 1

Aggregation level	1	2	3	4
Determination of detectability for action potential	A	B	C	C

[0075] As a result, it was clarified that when nerve cells evaluated as Aggregation Level 1 are used, the action potential can be detected well.